

What is claimed is:

✓  
1. A method for determining the activity of a protease, said method comprising

5 a) incubating a mixture of said protease and a substrate capable of being bound to an anchor, said substrate having a fluorescent radical attached thereto;

b) binding the substrate to an anchor;

10 c) measure the fluorescence polarization of the mixture.

2. The method of Claim 1 wherein the substrate is selected from compounds of Formula I

15 
$$Z-(W)_m-X-(V)_n-Y \quad (I)$$

wherein X is an amino acid sequence sufficient for substrate recognition by a protease; wherein V and W are independently selected from aminoalkylcarboxylic acids; wherein m and n are  
20 numbers independently selected from 0 and 1; and wherein one of Y and Z is a fluorescent radical and the other is a binding radical.

3. The method of Claim 2 wherein X is a peptide  
25 containing six to sixteen amino acids, inclusive; and wherein V and W are independently selected from glycine, 4-aminobutyric acid, 5-aminopentanoic acid, 6-aminocaproic acid and 7-aminoheptanoic acid.

30 4. The method of Claim 3 wherein the anchor is selected from a biotin selective protein, a solid support, and an antibody; wherein the binding radical is selected from biotin, digoxigenin and radicals capable of binding to a solid support; and wherein the fluorescent radical is  
35 selected from derivatives of fluorescein, rhodamine,

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Sub.C1

coumarin, eosin, pyrene, quinoline, DANSYL, dinitrophenyl, benzimidazole, DABCYL, EDANS, cascade blue, Texas red, acidine orange and BODIPY.

Sub.C1  
5                    5. The method of Claim 4 wherein the fluorescent radical is a fluorescein derivative.

Sub.C1  
10                   6. The method of Claim 5 wherein the biotin selective protein is avidin or streptavidin; wherein the binding radical is biotin; and wherein the fluorescent radical is DTAF.

15                   7. The method of Claim 1 wherein the proteases are viral proteases.

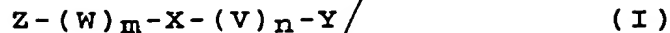
Sub.C1  
20                   8. The method of Claim 7 wherein the proteases are selected from HIV proteases and herpes proteases.

Sub.C1  
25                   9. The method of Claim 8 wherein the herpes viruses proteases are selected from HCMV proteases, MCMV proteases, HSV-1 proteases and HSV-2 proteases.

Sub.C1  
30                   10. The method of Claim 6 wherein the substrates are selected from biotin- $\gamma$ -Abu-Gly-Val-Val-Asn-Ala-Arg-Ser-Leu-Lys(DTAF)-NH<sub>2</sub> [SEQ ID NO:3] and biotin- $\gamma$ -Abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DTAF)-NH<sub>2</sub> [SEQ ID NO:4].

35                   11. A method for identifying compounds which inhibit a protease, said method comprising a) incubating a mixture of said protease, the compound, and a substrate having both a fluorescent radical and a radical capable of binding to an anchor; b) binding the substrate to the anchor; c) measure the fluorescence polarization of emitted light; and d) calculating the amount of protease inhibition.

12. A compound of Formula I



5 wherein X is an amino acid sequence sufficient for substrate  
recognition by a protease; wherein V and W are independently  
selected from aminoalkylcarboxylic acids; wherein m and n are  
numbers independently selected from 0 and 1; and wherein one  
10 of Y and Z is a fluorescent radical and the other is a  
binding radical.

13. The compound of Claim 12 wherein X is a peptide  
containing six to sixteen amino acids, inclusive; wherein V  
and W are independently selected from glycine, 4-aminobutyric  
15 acid, 5-aminopentanoic acid, 6-aminocaproic acid and 7-  
aminoheptanoic acid; wherein the binding radical is biotin;  
and wherein the fluorescent radical is a fluorescein  
derivative.

20 14. The compound of Claim 13 which is biotin- $\gamma$ -Abu-  
Gly-Val-Val-Asn-Ala-Arg-Ser-Leu-Lys(DTAF)-NH<sub>2</sub> [SEQ ID NO:3].

25 15. The compound of Claim 13 which is biotin- $\gamma$ -Abu-  
Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DTAF)-NH<sub>2</sub> [SEQ ID NO:4].

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